XLVII.—*Further Observations on* Amœba villosa *and other indigenous Rhizopods.* By G. C. Wallich, M.D., F.L.S., F.G.S., &c.

[Continued from p. 371.]

[Plate X.]

Before proceeding to offer a few general remarks on the relations of *Amœba villosa,* and two other forms obtained from the same locality, with the rest of the group to which they belong, it is desirable that I should adduce some supplementary observations made by me during another month's study of these organisms.

In many specimens of the *Amœba,* and more especially such as appear to be in full vigour of growth, the protoplasm is densely charged with certain granules, of larger size than those mere points which pervade it under all circumstances, but nevertheless extremely small. If examined cursorily, these granules may readily be mistaken either for extraneous bodies derived from without, or for mere consolidated particles of the sarcode itself. They are so minute as to render it difficult to trace any difference in their dimensions, when seen under the medium powers of the microscope. But their aspect when isolated along with a thin film of their sustaining protoplasm, and examined under a power of from four to five hundred diameters, at once led me to suspect their crystalloid character; and this view was fully borne out on submitting them to a still higher degree of magnifying power; for whilst no analogous bodies, or, indeed, any insoluble saline particles, were discoverable in the material in which the *Amœbæ* were contained, these crystalloids proved to be distinct rhombohedrons possessing a higher index of refraction than water. They measure in length from $\frac{1}{15000}$th to $\frac{1}{4300}$th of an inch, with a breadth of from $\frac{1}{20000}$th to $\frac{1}{5000}$th of an inch. Their minuteness renders the precise definition of their angles a matter of considerable difficulty; but nevertheless, by taking the mean of a number of measurements, I found the more obtuse angle to be about 140°. Hitherto I have been unable to determine their nature by chemical tests, further than discovering that when a slide, on which a number of the *Amœbæ* have been dried, is treated with dilute hydrochloric acid, all trace of the crystals is entirely lost. Coupling their rhombohedral figure with this fact, it seems probable that they may consist of carbonate or some other salt of lime. This, however, is a point demanding further careful investigation. I may mention that precisely similar crystalloids have also been detected by me in the sarcode of *Euglypha, Arcella,* and *Acanthometra.* (See Pl. X. fig. 7.)*

* As is well known, "prismatic crystals" were observed by Huxley in *Thalassicolla.*

In addition to these crystalloids, which seem to occur more largely in some individuals than in others, two other kinds of corpuscles are to be found. Both have a spherical outline, and are not discoidal, as may be seen on watching them roll over when at the immediate extremity of a pseudopodium. They vary from $\frac{1}{3300}$th to $\frac{1}{1600}$th of an inch in diameter, are devoid of any appreciable cell-wall, even when examined under the higher powers of the microscope, and are formed of a peripheral layer of pale, nearly colourless, granular protoplasm surrounding a clear fluid centre, the average diameter of which is about $\frac{1}{10000}$th of an inch (fig. 5). To these bodies (which are in all probability identical with the "discoid ovules" described by Carter, in his admirable observations on the Organization of the Infusoria of Bombay *, as occurring in *A. Gleichenii*, and as also seen in *A. verrucosa*†) I have applied the term nucleated *corpuscles*, as not involving a function which must still be regarded as only hypothetical, although I fully agree with Mr. Carter in the view that these bodies perform some important part in the process of reproduction. The second kind of spherical corpuscle to which allusion has been made, although not nucleated as in the former case, appears destined to exercise the function of a true ovule in some of the Rhizopods, if not in all, as shall presently be shown; and therefore, taking into consideration the very marked resemblance of the latter, in all save the trivial item of depth of colour, to the "yellow bodies" met with in the Foraminifera, Polycystina, Thalassicollidæ, Acanthometrina, and two new families I propose to establish for the reception of certain allied but heretofore imperfectly understood pelagic genera, it appears absolutely necessary to distinguish between the two kinds of structure.

To these bodies I have accordingly given the name of *Sarcoblasts*. In *Amœba* they are somewhat larger than the nucleated corpuscles, being from $\frac{1}{2000}$th to $\frac{1}{1650}$th of an inch in diameter. In their earlier stages they present a faint yellow tint, are somewhat oily-looking, but afterwards become almost colourless. They are distinctly granular, nearly homogeneous throughout, and, like the corpuscles, devoid of cell-wall (fig. 6). Both, however, are distributed equably through the endosarc, and take an equal share in the pseudocyclosis which involves all foreign matters and, under certain circumstances, the nucleus, contractile vesicle, and vacuoles.

There seems reason to believe that all organic substances intended for food are invariably subjected to the digestive process through the medium of vacuolar cavities specially extemporized

* Ann. Nat. Hist. ser. 2. vol. xviii. pl. 5. fig. 5. † *Ibid.* vol. xx. p. 37.

for their reception. In the case of insoluble substances—such as particles of mineral matter, many of which effect an entrance into the endosarc of the Amœbans, either along with alimentary matter or accidentally—the vacuolar cavity is not necessarily present, but the atom simply rests within the protoplasm. In *A. villosa* the food-vacuoles are generally observable, although with difficulty when the body is much distended by extraneous substances and endogenous organic granules. In some specimens they are very strikingly developed. For these vacuoles I propose the term *food-vacuoles,* in contradistinction to the simple vacuoles which form and disappear spontaneously within the protoplasmic substance and, when in such great numbers as to impart an almost parenchymatous character to portions of the structure, apparently forebode its disruption or death.

From the manner in which the food-vacuoles are formed at the surface—in *Amœba* by the coalescence of pseudopodia which envelope the object about to be incepted, and in *Actinophrys,* by the projection of a coarse irregular network of ectosarc, aided by the coalescence of the pseudopodia in the immediate vicinity—it would appear that a certain quantity of the surrounding water is always admitted into the newly formed cavity. This water is probably essential to the due performance of the digestive process, and in part enters into the composition of the alimentary fluid. On the other hand, it is probable that, in certain cases, a portion of the water becomes absorbed by endosmosis, without undergoing chemical change, more especially where the food-particles have been completely dissolved through the digestive process. In this condition, the vacuoles may frequently be seen sharing in the pseudocyclosis, till by slow degrees they entirely disappear,—the effete watery particles, as I have already stated, being then poured exosmotically into the contractile vesicle, and ultimately discharged.

In my previous notice I drew attention to the highly developed membranous capsule within which the nucleus of *A. villosa* is contained. According to Carpenter (the latest authority on the subject)*, the nucleus of *Amœba* presents "the aspect of a clear flattened vesicle surrounding a solid and usually spherical nucleolus, and is adherent to the inner portion of the ectosarc, and projects from it into the general cavity." Carter also describes it "as an organ situated on the outer portion of the sarcode, which, when well marked, presents under the microscope the appearance of a full moon (to use a familiar simile), with similar slight cloudiness." He adds, "It is discoid in shape, of a faint yellow colour, and fixed to one side of a trans-

* Introduction to the Study of the Foraminifera, London, 1862, p. 24.

parent capsule, which, being more or less larger than the nucleus itself, causes the latter to appear as if surrounded by a narrow pellucid ring"*.

Now, whilst both these observers speak to a vesicular boundary to the nucleus, it is evident, I think, that they do not allude to that highly specialized membranous covering which is so remarkably manifest in *A. villosa*, and is actually separable from the body. The point is not so immaterial as it would seem to be at the first glance, inasmuch as a definite vesicular covering has also been supposed by Claparède and others to appertain to the contractile vesicle of *Actinophrys*, whereas, as I shall presently endeavour to show, none is in reality present either in the contractile vesicle of that genus or of *Amœba*; and hence the character becomes to this extent a distinctive one. Moreover it is important from its at once stamping as complete the analogy I desire to draw between the nucleus of *Amœba* and of *Plagiocantha*, *Thalassicolla*, *Acanthometra*, and *Dictyocha*, whereby the relation of the several parts to each other in these genera although belonging to a distinct order, becomes intelligible.

The position of the nucleus in *Amœba villosa* has already been shown to be variable. I am not aware that, under any circumstances, the nucleus of this form can strictly be said to adhere to the inner portion of the ectosarc, as indicated by Carpenter, or to be situated on the outer portion of the endosarc, as stated by Carter—its temporary location in the vicinity of the villous patch, spoken of in my former paper, being due, as I conceive, to that more highly differentiated condition of the posterior part of the organism which constitutes so striking a feature in *A. villosa*. I have as yet been unable to ascertain positively the manner in which both nucleus and contractile vesicle are periodically sustained near the villous patch; but, from the constricted shape frequently assumed in this region, and the tendency to project the pseudopodia principally from the advanced or anterior portions of the body, there can be little doubt that it is brought about by the augmented contractile power of the posterior extremity. On the same hypothesis we may account for that peculiar state in which a mass of granular matter, resembling

* On the Organization of the Infusoria, Ann. Nat. Hist. ser. 2. vol. xviii. p. 221.

Mr. Carter is of opinion that there is a central cavity in *Amœba*, at times distensible with water. Thus he speaks of an "Amœbous cell under spherical distension." This may account for the view held by him with regard to the position of the nucleus in the Bombay form; but I must distinctly observe that no such cavity is present in *A. villosa*. (Ann. Nat. Hist. vol. xviii. p. 223, and explanation appended to fig. 1. plate 5, accompanying his paper.)

that of the true nucleus, and which is derived in all probability from the sarcoblasts already referred to, becomes first aggregated in that region. It only remains to be further noticed regarding the nucleus, that in that condition of the organism in which a very limited quantity of foreign matter is present, and the crystalloids, nucleated corpuscles, and sarcoblasts are most abundant, this organ frequently undergoes subdivision—two separate nuclei occurring under these circumstances, and sometimes, but much more rarely, three. Endogenous subdivision (that is, endogenous with reference to the capsule) does not take place in such cases, but nucleus and capsule both undergo complete and simultaneous binary division. I am unable to say whether, in those examples that present three nuclei, the whole of these become separated by one duplicative act or by two. It has already been shown that where the multiple nuclear bodies supervene on the accumulation of granular matter at the posterior portion of the *Amœbæ*, I failed to trace any capsular coverings. It seems probable, therefore, that, in the former case, the process may be regarded as one of simple fission, in the latter, of molecular segregation*. But on this head anything like positive information is still wanting.

Great diversity of opinion seems to exist amongst naturalists not only regarding the office, but also the actual structure of the "contractile vesicle." That misapprehension should exist on the subject is by no means surprising, however, when we bear in mind that the name has been indiscriminately applied both to the rhythmically contracting organ of the Rhizopods and of the true Infusoria, and that, under the supposition that identity of action involves identity of conformation, a true vesicular wall has been assigned by several of our most eminent writers to the pulsating cavity of the former group of organisms. Without entering at present upon the question whether the Rhizopods ought to be regarded as unicellular, multicellular, or altogether devoid of cell-structure, I would observe that, if the presence of such structure within the substance of certain members of the Amœban group has not been already demonstrated by the perhaps less definite examples adduced by Carter and others, the indisputable envelopment of the nuclear body of *Amœba villosa* by a distinct membranous capsule at once settles the point. But, on the other hand, my own observations tend to prove that this structure is only to be met with in the two higher orders of the Rhizopods, and not in the lowest order, which, according to

* Carter describes a somewhat similar process as occurring in *Amœba radiosa*, and "ending in the production of a mass of spherical, delicate, transparent, granuliferous cells." (See his paper on the Organization of Infusoria, Ann. Nat. Hist. ser. 2. vol. xviii. p. 225.)

my arrangement, comprises the Gromida, Foraminifera, and Polycystina. In these families, the nuclear granules are diffused, and assume the multiple character of sarcoblasts, which, on separation from the parent sarcode, constitute the rudiment or "primordial segment" of the new brood.

In my experience, the contractile vesicle does not make its appearance either in the HERPNEMATA (which constitute the first or lowest order) or the PROTODERMATA or second order (which comprises the Thalassicollidæ, Acanthometrina, and their allies), but occurs, for the first time, in the third order or PROTEINA, in which I associate the *Actinophryna*, *Lagynidæ*, and *Amœbina*—the name of this order being adopted from the classification of MM. Claparède and Lachmann, who, in like manner, associate *Actinophrys* and *Amœba*, but on widely different grounds from those that have led me to assume their ordinal unity.

In the third order both nucleus and contractile vesicle, I believe, are invariably present, although naturally difficult of detection in the testaceous genera. The latter organ, however, in so far as my experience of living representatives of nearly every important form enables me to arrive at a correct opinion on the subject, ought not to be regarded as a definite-walled contractile sac, distinct in composition from the remainder of the protoplasmic matter, but simply as a specialized vacuolar cavity *formed out of a portion of the ectosarc.*

Mr. Carter, in his paper on the Organization of the Infusoria, already referred to (*l. c.* p. 130), says, "That the vesicula is a distinct organ, and not merely a space like the digestive globule, might be inferred from its always appearing in the same place in the same species"*. For reasons already adduced, I am inclined to regard it in an opposite light—that is to say, as merely a space bounded by a layer of ectosarc, and not by a membranous wall of distinct origin and character. And I think my view will be at once recognized as correct when it is taken into consideration that we constantly see multiple contractile vesicles, not only already formed, but actually forming under our eyes and again

* On reference to what has been stated in a preceding page, it will be seen that Dr. Carpenter speaks of the nuclear capsule as "a clear flattened vesicle;" whilst he considers the contractile vesicle to be "a vacuole with a defined wall" (Introduction to the Study of the Foraminifera, p. 14). Mr. Carter, again, describes the nucleus as discoid in shape, and fixed to one side of a transparent capsule, whereas he refers to the contractile organ as "distinct and not merely a space like a digestive globule." In directing attention to these definitions, I am desirous of showing that both these authorities express their opinions on the subject with a degree of reserve which was fully warranted under the circumstances, but which leaves the proofs as to the existence of a true membranous vesicle in one case dependent on equal proofs of its existence being forthcoming in the other.

coalescing to constitute a single cavity, or, as happens occasionally, each undergoing a separate systole. This I maintain could not possibly take place except under the conditions I have endeavoured to describe.

The vacuoles within which organic substances, or animalcules, incepted for food become amenable to the digestive process are similarly constituted; and if what has been advanced by me in my previous paper be correct (that is to say, if endosarc and ectosarc are not permanent portions of the Rhizopodal structure, but mutually and temporarily convertible one into the other), it is manifest that the higher state of differentiation exhibited by the vacuolar wall is wholly due to a like cause, namely, contact with a portion of the surrounding fluid.

I am well aware that the permanently visible villous area of the Hampstead *Amœba* appears to militate in some measure against the universal correctness of this hypothesis. But I have already stated that this species can hardly fail to be regarded as embodying the highest type of Rhizopod life, and as bridging over the hiatus between true Rhizopod and true Infusorial organization. If a boundary exists at all between these two great groups of the Protozoa, it will, I think, be found to consist in this—that whereas in the Rhizopods there is no permanent orifice for the inception and extrusion of foreign or effete matter, and the endosarc and ectosarc are not permanent portions of the organism, but, as already maintained, mutually convertible one into the other, in all mature Infusorial forms permanent orifices occur for the inception and extrusion of such matter, and there is no convertibility of parts once established*. Hence, even granting, for the sake of argument, that the villous patch in *Amœba villosa* is not only permanent in position as regards the rest of the body, but, in a like sense, permanent in composition during the entire period of the individual's existence, I contend that we should not be warranted, on this ground alone, in pressing such an objection. But I am by no means prepared to allow that such a permanent condition of the villous region does exist, inasmuch as it appears to me to be far more probable and conformable with the phenomena referred to, to assume that a slow but constant interchange of protoplasmic matter takes place there, as it does, although more rapidly and perceptibly, in the other portions of the structure.

During the bygone month I have seen numerous examples of the infundibuliform excretory tubule of *A. villosa*. But I have likewise been able to satisfy myself that this tubule is an extem-

* *Asplanchna* furnishes no valid objection to this generalization, even if we hesitate to accept the most recent views promulgated regarding its structure; for one orifice may serve both purposes.

porized part of the organism, and that, in many cases, the layer of protoplasm which constitutes it during the extrusion of effete matter, and occasionally also of minute but perfectly formed *Amœbæ,* is actually disengaged along with the object whose egress from the main body it presides over and probably effects; so that even here we encounter phenomena which, although as yet inexplicable, tend directly to prove the accuracy of the views referred to. In short, the vacuolar sac becomes the tubule, being in some cases reabsorbed into the substance of the body, in others actually expelled entire, as shown in Pl. IX. figs. 3 & 4.

Again, it appears to me that, assuming the hypothesis to be admissible, we not only render intelligible the mysterious and otherwise inexplicable properties of sarcode, but find a clue to the determination of the function performed by the contractile vesicle.

I am able fully to confirm the statement of Carter that this body invariably discharges itself externally in the Rhizopods, although aware that this view is opposed by M. Lachmann and others. The orifice through which the discharge of its watery contents is effected is not of a permanent nature, but, like the tubule occasionally seen in the region of the villi, comes into existence only under the operation of the force that distends the wall and eventually bursts it. We frequently see that the systole of the vesicle is interrupted before the entire obliteration takes place, which most commonly occurs. But it is a mistake to suppose that the circular outline then left, and which forms the basis, as it were, of the vesicle when renewed, represents the orifice by which the contained fluid escaped. That orifice we can very rarely detect, even under the highest powers of the microscope*. I can personally speak to its subtle but nevertheless appreciable character, having watched its action for a considerable period on two occasions, in Bengal—namely, in an *Amœba* closely allied to, if not identical with, *A. villosa,* and in a *Kerona,* its distinctness in the Infusorial animalcule being only rendered greater by the greater ease with which the latter was maintained in the position best fitted to carry on the observation.

Mr. Carter, in describing the contractile vesicle (*loc. cit.*), says it is neither a circulatory nor a respiratory, but an excretory organ, and, referring more particularly to *Amœba* and *Actinophrys Sol,* he adds,—"During the act of dilatation, the vesicula projects far above the level of the pellicula, even so much so as

* I would distinctly guard myself against appearing to convey the idea of a *valvular* opening such as is supposed to exist by Weston in *Actinophrys Sol* (Quart. Journ. Micr. Soc. vol. iv. p. 116).

occasionally to form an elongated, transparent, mammilliform eminence, which, at the moment of contraction, subsides precisely like a blister of some soft tenacious substance that has been pricked with a pin"*. Mr. Carter does not mention any special excretion—that is to say, whether he means the excretion of effete nutritious matter or water only. If the latter, I entirely agree with him—my view being that, whilst the general substance of the body absorbs water from the surrounding medium by endosmotic action, and partly also by admission *en masse* into the extemporized food-vacuoles; through the agency of the opposite or exosmotic action the fluid is poured into the contractile vesicle, gradually distending it, as described, until rupture ensues. The moment that the tension on the most prominent and, consequently, the most attenuated portion of the vesicle is relieved by the escape of its contents, the orifice becomes obliterated by the union of its edges, and the process is repeated. In this sense, then, the contractile vesicle may be regarded as a true water-vascular and excretory organ. I need only add that, according to my own experience, and in accordance with the opinion expressed by several eminent observers, the contractile vesicle takes no share, under any circumstances, in the capture, inception, or extrusion of any solid substances.

In a former page, allusion has been made to a mode of reproduction which, although closely bordering on simple gemmation, must, I think, rather be regarded in the light of viviparous parturition. I had never noticed it prior to my recent and almost continuous observation of the Hampstead *Amœba*, nor am I aware that it has previously attracted the attention of other naturalists, although M. Jules Haime records examples amongst the Infusoria in which minute bodies ejected from the body of the parent have become converted into young animals whilst still under observation†. In *Amœba*, however, such an oversight may easily be accounted for by the circumstance that the newly liberated individuals are so minute, in comparison with the parent form, as to be barely distinguishable unless examined under high powers and with a knowledge of their origin. They rarely measure more than $\frac{1}{2500}$th to $\frac{1}{1660}$th of an inch in their most extended state, and yet, when carefully analysed, exhibit nucleus, contractile vesicle, villous tuft, and even protoplasmic granules, with every distinctive character discernible in the parent from which they sprang. It is also a very significant and remarkable fact, that, even in this minute stage of their existence,

* Ann. Nat. Hist. ser. 2. vol. xviii. p. 126.

† Carter on the Organization of Infusoria (Ann. Nat. Hist. ser. 2. vol. xviii. p. 223.

they throw out pseudopodial processes so various in outline that, were it legitimate to base specific distinctions on such variations, we might have nearly every form heretofore regarded as specifically distinct by some observers produced from one parent source. Indeed, coupling this fact with others bearing on the same subject, although not prepared to affirm that the whole of the varieties of *Amœba* are reducible to a single primary specific type, I candidly confess that the balance of evidence appears to me to point towards such a conclusion, and to indicate that the divergences in form and outward characters may be wholly dependent on the local and even temporary conditions of the medium in which the young animal happens to make its appearance in the world.

It is one of the most perplexing accompaniments of microscopic research, that, in addition to the ordinary difficulties attending the study of the reproductive phenomena in organisms which admit of observation by the unaided vision or with the aid of low magnifying powers, the chances are greatly against our having the object under our eye at the exact moment that the phenomena are taking place which we desire to witness. From the extreme rapidity with which they are sometimes completed, compared with analogous processes in the higher orders of being, this result is scarcely surprising, even if we treat lightly the difficulty inseparable from the survey of vital actions on so minute a scale. On the other hand, there is reason to fear that erroneous interpretations have often been put upon microscopic phenomena in consequence of a failure on the part of the observer to watch them from their commencement to their termination*. The following instances, which are not the only ones that have presented themselves to my notice during my recent close scrutiny of the indigenous Rhizopods, will prove the truth of this remark.

Fig. 11 represents an abortive effort at division taking place in a specimen of *Amœba radiosa*. It will be seen that nothing could be less conformable with the published descriptions and figures of that form than the individual here portrayed. But nevertheless I can vouch for its being the form which has been so named, not only from the fact of the locality in which it was found containing numerous specimens unmixed with

* The drying up of the minute portion of water in which living organisms are being submitted to long-continued observation under the microscope may be very successfully obviated by resting the slide, when not actually required, across the mouth of a wine-glass containing water, and carefully placing a strip of fine calico across the thin cover, with its ends hanging down into the fluid in such wise as to allow capillary attraction to do all that is requisite.

other varieties, but from the specimen itself having ultimately assumed all the characteristics of *A. radiosa* immediately after the termination of the appearances depicted in the figure.

Judging from the appearances at first presented, I naturally expected the occurrence of binary division—two lobes instead of three being then only visible; and accordingly I directed special attention to the share taken in the process by the nucleus and contractile vesicle. But these bodies gave no sign of participation beyond passing several times, during the ordinary contractile movements of the lobate masses, through the connecting isthmuses, either into the same or into separate lobes. No pressure was exerted, nor was the form assumed due to the juxtaposition of foreign matters. Nevertheless fission did not take place; and after an hour's apparently incessant struggle to part company, during which period the lobes and isthmuses did not materially alter their relative proportions, the three portions gradually coalesced, and the specimen moved away energetically, putting forth the tapering and radiate pseudopodia supposed to be distinctive of *Amœba radiosa*.

It is no doubt true that the unity of the nucleus may have interfered with the consummation of the process. But here, again, generalization fails to some extent; for on two occasions I have seen *Amœba villosa* divide without the nucleus being involved. In both cases the villous patch was nearly equally parted; only that half of the body, however, which retained the nucleus moved about vigorously and exhibited the typical characters, whilst the other half assumed a spherical shape, and merely oscillated very slowly and steadily to and fro on the same spot, without projecting pseudopodia, or materially altering its outline.

In these examples, also, all undue pressure was avoided; and the extrusion of nearly the whole of the effete alimentary particles by each half—which I have frequently found to be the precursor of the process of fission—took place almost at its commencement.

The third example I have to mention occurred in a large specimen of *Actinophrys Eichhornii* which I disengaged from the side of the vessel to which it was adhering, and carefully placed for observation in a watch-glass. When removed, it was apparently undergoing the common process of binary division. At all events, whether that process was going on or the case was one of "zygosis" or amalgamation of two individuals, it is quite certain that there was a partial, but nevertheless effective, fusion of the sarcode substance at the constricted portion. In this species, as shall presently be shown, the nuclear bodies are small and, generally speaking, multiple. Hence no information was derivable from their distribution. The contractile vesicles were multiple also, two being distinctly visible, and in regular rhythmical action along the peripheral plane of each half; whilst others may have been present, but obscured on the upper or under surfaces of the structure. When first seen, the well-marked peripheral layer of protoplasmic cellules belonging to each half was uninterrupted at the constricted portion; and around the latter was a somewhat irregular zone of bubble-like protoplasm, which merged into that of the masses on either side of it. But soon the constriction became more and more complete; and after a time the two halves were held together only by a narrow isthmus of sarcode. At this stage, however, a retrogressive action commenced, and ultimately the two portions became fused into a single *Actinophrys*. No movement was observable except on the systole of the contractile vesicles, when the half on which the vesicle was situated oscillated slightly, but very perceptibly. I have only to add that no other specimen was placed on the watch-glass during these appearances, which lasted over a period of four hours. The single specimen then measured $\frac{1}{70}$th of an inch in diameter independently of the pseudopodia.

These examples are instructive for two reasons: firstly, because they tend directly to confirm the statement originally put forward by Schneider with reference to the occasional zygosis or coalescence of two previously distinct individuals; and secondly, because they indicate how much caution ought to be exercised before an opinion is pronounced upon the nature of phenomena the order of which has not been followed from their commencement to their termination*.

It now remains for me to direct attention to forms of *Actino-*

* In my notes on the presence of animal life at great depths in the ocean (published in November 1860), and also in a paper in the 'Annals and Magazine of Natural History' for July 1861, I directed attention, for the first time, to the occurrence of the Coccoliths (minute discoidal structures previously detected by Huxley in the material of the soundings, but regarded by him as inorganic) in spherical cells, to which I accordingly applied the name of Coccospheres; and I further pointed out that as entire Foraminiferous shells, and more especially those of *Textularia* and *Rotalia*, are frequently met with in the soundings wholly made up of segments resembling these Coccospheres in every particular, these bodies would appear to be connected with the reproductive process. Although I have not had the opportunity of tracing the actual sequence, I think it highly probable that the Sarcoblasts, to which I have alluded in a former page, first become Coccospheres, and are then developed into the perfect shell by the ordinary process of gemmation. Here, then, is a case in which the difficulties attending the study of the reproductive phenomena in the Rhizopods are yet further enhanced. I may take the opportunity of stating that I have recently met with Coccospheres in great abundance in dredgings from the English Channel. The means of clearing up the point are therefore at hand.

phrys and *Difflugia* (also from Hampstead) which, although not specifically new, offer some important and, if I mistake not, hitherto unnoticed characters.

As is well known, in *A. Sol* the body consists of a spherica or nearly spherical mass of sarcode, the external layer of which is said to be permanently distinct from the endosarc, notwithstanding the admission that no definite boundary-line is traceable between these two portions of the structure, and that they insensibly merge one into the other. Carpenter* describes it as follows:—"The pseudopodia seem to be derived from the ectosarc alone, the endosarc not extending itself into them. They possess, moreover, a degree of consistence which usually prevents them from coalescing when they come into contact with one another; and whenever such a coalescence does take place, it is to a much smaller extent than is common among Foraminifera." And again, "Although the existence of a nucleus in *Actinophrys* has been denied, its presence (in certain species at least) must be regarded as a well-established fact." Speaking of the inception of food, he says, "The body taken in as food is received into one of the vacuoles of the endosarc, where it lies, in the first instance, surrounded by liquid." .... "Several vacuoles may be occupied at one time by alimentary morsels; frequently from four to eight are seen thus filled, and occasionally ten or twelve, Ehrenberg having in one instance counted as many as sixteen."

From what has been already advanced by me with regard to *Amœba*, it almost follows that I should view with extreme doubt the specific value of the characters assigned by different writers to the various forms of *Actinophrys* that have been described as distinct; and, in addition to the reasons I shall adduce in support of this view, I would call attention to the indirect evidence of its correctness afforded in the errors of identification committed by some of the most acute observers with regard to the forms looked upon as the most persistent and definite. Thus Kölliker mistook *A. Eichhornii* for *A. Sol.* Claparède wrote a long paper on *A. Eichhornii*, and afterwards discovered he had been describing *A. Sol*†. Perty is of opinion that *A. Eichhornii* is an enlarged state of *A. Sol*, whilst Stein also affirms that *A. Eichhornii* is no other than the latter species. I have only to observe on this head, that it would indeed be surprising if the confusion thus created were one whit less than it is, where such characters as the length of the pseudopodia, the diameter of the

* On the Study of the Foraminifera, p. 18.

† See Prichard's 'Infusoria,' 4th ed. p. 560, and M. Claparède's paper in the 'Annals,' 2nd ser. vol. xv. pp. 211 and 285. These examples might, however, be multiplied.

body, the perfectly regular or irregular outline of the latter, and even trifling modifications in its colour, have been accepted as specifically valid*.

Why, then, it may be asked, have I referred the Hampstead form to *A. Eichhornii*? I reply, solely because the characters presented by this variety are those which appear to me to illustrate in the same individual the true offices and relations of the several parts of the Actinophryan structure to each other and to allied genera, and ought therefore to be regarded as typical of that genus.

The Hampstead form, like the *Amœba* already described, is unusually large, at times attaining a diameter of $\frac{1}{30}$th of an inch, irrespectively of the pseudopodia. Whilst I write, I have a number of living specimens before me, obtained two months ago, in which the entire structure is so pellucid and definite that it can be resolved with a common pocket-lens. The shape is spherical, not discoidal, under ordinary conditions, even when the creature is adherent to the sides of the glass vessel in which it is contained,—this being reconcileable with a fact I can attest, namely, that the surface of the body does not come in contact with the glass, but is entirely supported by the tenacity of the intervening pseudopodia. The sarcode substance is colourless, as is the sarcode of all *Amœbans* and *Actinophryans*, except under abnormal circumstances. The pseudopodia are sometimes long, sometimes short; at times perfectly rigid and smooth, at times slightly tuberculated and sinuous. When rigidly extended, they never coalesce; when bent and supple, and more especially when about to encircle some food-particle in their inevitable embrace, they coalesce as freely as those of the Foraminifera. Now and then, but rarely, the vacuolation, so universal and marked in the form as it most constantly occurs at Hampstead (fig. 1), is partially superseded by the coalescence of a number of the cell-like cavities, and the ectosarc or endosarc exhibits the aspect of ordinary unvacuolated protoplasm, and we may more legitimately apply the terms "medullary" and "cortical" to the inner and outer portions of the organism. But physiologically there exists no such permanent distinction of parts. There is invariably a line of demarcation between the "cortical" and "medullary" portions; but the most careful analysis of the structure, even when assisted by the highest powers of the microscope, does not enable us to detect the

* If the characters of *A. oculata* are correctly drawn, and if, as asserted, food does not pass into the "medullary" substance, but remains during the assimilative process within the "cortical" layer, that form must not only be regarded as specifically distinct, but as presenting a feature which is quite anomalous in the group to which it belongs.

slightest appreciable difference between the intimate texture and composition of the two parts, the figure of their polygonal cavities, the proportional thickness of the walls of the latter, their optical characters, or the capability of the two portions to coalesce *,—the fact being that each polygon is essentially composed of both endosarc and ectosarc, the latter being the necessary result of the contact of its internal surface with its fluid contents, which do not consist principally of protoplasm, but of water; whereas the former may be said to occupy the interval between the wall of adjacent cavities, and is actually distinguishable by its finely granular and viscid appearance wherever fusion or coalescence has taken place to a certain extent. The pseudopodia and slightly thickened peripheral layer are also finely granular. The former are given off from the external surface, it is true; but, on the other hand, the walls of the polygonal cavities present no appreciable differences in character from the pseudopodia, beyond being flattened instead of filamentous expansions of the same tissue. And, lastly, I would lay special stress on a phenomenon which this form of *Actinophrys* constantly enables us to witness, namely, the absorption of the vacuolar food-cavities, *formed at the immediate surface of the organism only at the period when required, and which are not previously existing and persistent portions of the creature*, as has been supposed. As in *Amœba*, the process of inception of food consists simply in the formation of an extemporized cavity, partly derived from the coalescence of the pseudopodia that have captured the object, partly from the portion of ectosarc that happens to be brought into contact with the object, and the subsequent elimination of the nutrient matter by the vacuole thus formed and now drawn into the centre of the body by the inherent contractility of the surrounding protoplasm. If this view of the phenomena be correct, we must either assume that a constant diminution in bulk of the ectosarc must take place at each formation of a food-vacuole, or admit what I contend for, namely, that for every portion of the outer layer, constituting the ectosarc for the time being, which is so removed from its position, a portion of the subjacent endosarc forthwith steps forward and fills up the vacant rank. As is well known, the organism captured for food is sometimes almost as large as the *Actinophrys* itself (see fig. 4). Unless my hypothesis be admitted,

* There appears no good ground for supposing that the vacuolation witnessed in *A. Eichornii* is anything more than an extreme example of what takes place frequently in *Amœba* to a great, but not so great an extent. In *Thalassicolla nucleata* we have a near approach to the same structure; and, as in the form under notice, the larger vacuoles are external, the smaller ones internal, with reference to each other.

if the vacuole for the reception of such particle is formed at the surface (and it unquestionably is so), there is nothing for it but to accord to *Actinophrys* the power of turning itself inside out after the fashion of *Hydra*. But even assuming this incredible explanation to be correct, we must remember that in *Hydra*, after the operation, the external surface becomes differentiated into the normal condition previously existing. In short, here, as in *Amœba*, the portion of protoplasm in immediate contact with the surrounding medium becomes *ipso facto*, and *for the time being only, ectosarc*; and on no other supposition is it possible rationally to account for the phenomena.

I have stated that in *Actinophrys Eichhornii* there is invariably present a line of demarcation between the external and internal—or "cortical" and "medullary"—portions of the structure. This is not produced by any difference in the intimate composition of these two portions, but is entirely dependent on the occurrence of a larger and more symmetrically arranged series of polygonal vacuolated cavities around a smaller and irregular central series. Owing to the uniform size of the former series, and the union of such of their polygonal planes as are nearest the centre of the body, the appearance of a distinct concentric ring is produced. Sometimes, however, more than one ring is observable. This is due to the formation of a second series of symmetrical cavities in the protoplasm.

My reasons have already been given for discarding the view that the outer and inner portions of *A. Eichhornii* represent the ectosarc and endosarc as separable from each other. On what then, it may be asked, does the peculiarity referred to depend? I would answer, on that manifest idiosyncrasy which in one case leads to the formation of a symmetrically sculptured test composed altogether of an exudation from the animal, and in another of a test in which the animal exudation shows no sculpturing, and is merely the basal matter into which mineral or other foreign particles are impacted. And, lastly, I need hardly remind the reader that, in the vegetable kingdom, we constantly meet with manifestations of a like idiosyncrasy, in similar lines of demarcation between the cells constituting the external and internal layers of a leaf or a stem.

Taking all these circumstances into consideration, I think there is sufficient ground for believing rather that *A. Sol*, *A. oculata*, *A. viridis*, and *A. Eichhornii* are varietal forms of the same species, at different periods of its history, or engendered by the varying conditions of the medium in which it is found, than that they are specifically distinct forms.

Allusion has been made to the multiple nuclei of *A. Eichhornii*. Before briefly describing their appearance, it is desirable that I should define the meaning of a term which has been so indiscriminately applied to the most widely differing portions of the Rhizopodal structure. Müller* uses the term at one time to signify the central or primordial chamber of the siliceous shell of the *Polycystina,* and at another to distinguish the more brilliantly coloured contents of that chamber from the otherwise identical sarcode of those portions of the structure that are subsequently developed. He also employs it in defining the central point of union of the siliceous framework of *Acanthometra.* Stein, it would appear, applies it to the entire "medullary" portion of *Actinophrys oculata,* which I regard as nothing more than a small form of *A. Eichhornii;* whilst Carter and Carpenter employ it in its only legitimate sense—that is, to denote a permanent part of the protoplasmic substance, more or less distinctly granular when fully developed, having a definite outline, contained within a definite-shaped cavity, often seen to undergo binary division whilst the rest of the body still remains entire, and apparently serving some important purpose in the reproduction of the individual. In the latter sense the term is used by me in these pages.

In *A. Eichhornii,* the subdivision of the nuclear body seems to keep pace with the extraordinary degree of vacuolation to which reference has already been made. For instead of meeting with it as a simple aggregated mass such as we find in *Amœba,* it is split up into numerous minute spherical masses, each of which presents the characters of a true nucleus on a reduced scale. These multiple nuclei are distributed, here and there, through the protoplasm—each occupying a spherical cavity which is completely filled up by the granular matter, and quite distinct in outward appearance from the polygonal soap-bubble-like mass of which the rest of the body is constituted. Facts are, however, still wanting to show whether the subdivision of the nuclei in *A. Eichhornii* is due to a repetition of the process which brings about the double or treble nucleus of the *Amœbæ,* or whether it is to be regarded as a normal and original condition in this form. If normal, it would certainly furnish a substantial character whereon to build a specific distinction. (See figs. 1 and 2.)

The hyaline transparence of the form under notice is admirably suited for affording an unobstructed view of the structure and mode of action of the contractile vesicles. As already stated, these vary in number. I have counted as many as five in the same specimen, all of which maintained a regular but perfectly independent rhythmical action. They never change their position, nor do they produce any appreciable effect on the cellular-

* Uber die Thalass. Polycyst. und Acanthom. des Mittelmeeres. Berlin, 1858.

looking protoplasm with which they are in immediate apposition, beyond what would be produced were they solid substances pressing mechanically on the structure with which they are in contact. As before stated, they never take part in the capture or inception of food, but continue their pulsations uninterruptedly even when in close proximity to a food-particle which is being dragged inwards. It is deserving of mention, that on the completion of the systole of the vesicle a depression or pit is temporarily formed, as shown in fig. 3, the surface of which is studded with villous corrugations not unlike those seen in the villous patch of *Amœba villosa*; and further, that, *previous to the actual contact* of the food-particle with the surface towards which it is being drawn by the cooperation of the surrounding pseudopodia, that portion of the surface, acting under an unexplained vital impulse, projects its irregular network of sarcode often far beyond the peripheral outline, to assist in building up the vacuolar cavity. This projection constitutes the "probosciform" apparatus of Ehrenberg.

Lastly, I have to mention the existence, in the majority of the polygonal cavities, of little clusters of the minutest granules, which during the vigorous condition of the organism are freely suspended by the watery contents, but fall down by their own specific gravity on the death of the creature, and rest passively on the most dependent planes of their polygons. Minute as are the crystalloids of *Amœba*, these are still more minute, and hence I have heretofore been unable to determine if they are of similar nature. However, from their appearance, their superaddition to the mere points that form an integral part of the protoplasm, and their uniform size, it is not improbable that these bodies will eventually turn out to be crystalloids also.

Rich as the Hampstead pools have thus been proved to be in facts illustrative of the life-history of the Rhizopods, many others remain to which my limits prevent any allusion being made on the present occasion. I must, however, briefly call attention to two forms of *Difflugia* that occur abundantly in the same material as the *Amœba*, and which, in like manner, have their tale to tell. In the variety depicted in fig. 12, the test is sometimes made up entirely, as there represented, of minute cylindrical pellets, probably chitinous, but certainly of animal origin. These are placed side by side in very regular order, resting, as it would appear, on a delicate and continuous membranous layer of the same substance. Sometimes, however, a part or the whole of these pellets is superseded by angular particles of sand. So far this form exhibits no novelty. But its new and unexpected feature consists in the mouth of the flask-shaped test being developed into a distinct septum and tube, whereby its character at once merges into that of a Dithalamous shell resembling those of certain Foraminifera. In crushed specimens the septal plate, and its aperture (which is situated at the dorsal side of the tubular expansion) may readily be seen. Fig. 13 exhibits a somewhat different shape, but no material difference in the composition or general character of the test, as is evident from the construction of that portion around the aperture, of chitinous pellets identical with those in the previously described variety. So that whereas in one form we observe a decided tendency to assume a character not ordinarily met with in any of the freshwater Rhizopods, in the other we have presented to us, in the same individual, a combination of characters on each of which it has been thought expedient by some writers to found as many distinct species. Nay, more than this, if the *principles* so admirably enunciated by Dr. Carpenter* are those on which alone a natural classification of the Rhizopods can be built up, we must at once and for ever discard as Ordinally distinctive those differences which do not involve the animal that forms the test, but only the test that is formed by it. It is, I conceive, impossible to examine the pseudopodia and other soft parts of *Arcella* and *Difflugia*, without at once perceiving their generic identity. It is equally impossible to examine those of *Euglypha*, *Lagynis* †, and some allied but less-known forms, without perceiving that the animals producing them are also generically identical. At most, mere modifications in the shape and proportionate quantities of the organic and inorganic elements that enter into the formation of the shell ought only to be employed to discriminate between species. But even here we may go a step too far, as is shown by the varieties of *Difflugia*, unless we start with the admission that the separation of such forms is simply a matter of convenience.

In conclusion, it only remains for me to state that, whilst courting the scrutiny on which the acceptance of every new scientific fact very properly depends, I have for the present purposely abstained from the extension of my hypothesis beyond those lowest forms of animal life to which reference has been made—my desire in so doing having been to dispel, with as little delay as possible, what I cannot regard otherwise than as a most unsatisfactory and untenable view of the mystery of Sarcode.

* Introduction to the Study of the Foraminifera.

† If we include those forms of decided *Euglyphæ* to which Schlumberger has given the distinct specific appellations of *Cyphoderia*, *Sphenoderia*, and *Pseudodifflugia*, the force of the observation becomes doubly manifest.

Errata in paper on Amœba *in the 'Annals' for May*: p. 366.

Twelfth line from bottom, *dele* "not."
Eleventh line from bottom, *instead of* "but is" *read* "and not."
Seventh line from bottom, *dele* "not."
Page 369, end of twentieth line from top, *dele* "comma."

EXPLANATION OF PLATE X.

Fig. 1. *Actinophrys Eichhornii*, showing the complete vacuolation of the "cortical" and "medullary" portions. A *Macrobiotus* and an *Astasia* are seen undergoing digestive absorption within the body, these organisms being enclosed within separate food-vacuoles. At *m* a second *Macrobiotus* is in the act of being drawn into the peripheral substance, and partially surrounded by the layer of sarcode which especially constitutes its special vacuole: *c v*, *c v*, contractile vesicles.

Fig. 2. A portion of the same individual, more highly magnified, in order to show more distinctly the vacuolation and polygonal character of the protoplasmic matter in this species of *Actinophrys*: *n*, *n*, nuclei; *m*, the *Macrobiotus* now completely enveloped by the layer of sarcode, and being slowly drawn into the endosarc; *f*, *v*, a food-vacuole, either after its contents have been altogether absorbed or after the excrementitious matter has been extruded. Both the above organisms are shown as focused down to a horizontal plane.

Fig. 3. Showing the villous appearance of the depression produced on the completion of the systole of the contractile vesicle.

Fig. 4. *Actinophrys Sol*, containing a large *Pinnularia* within a food-vacuole: *o*, *o*, oily globules within the protoplasm of the latter. This specimen, which was obtained from Hampstead also, is figured with a view to show how impossible it would be to distinguish it from an *Amœba* when, as often happens, the pseudopodia are entirely retracted. The food-vacuole was here very distinct.

Fig. 5. Nucleated corpuscles of *Amœba villosa*.

Fig. 6. Sarcoblasts or granular corpuscles of the same.

Fig. 7. Rhombohedral crystalloids of the same.

Fig. 8. Detached gemmule of the same, after the pseudosegmentation of the granular protoplasm of which it is composed; *p*, its mamilliform process.

Fig. 9. A young *Amœba villosa*, supposed to be the advanced stage of the gemmule, fig. 8: *a*, its villous tuft; *c v*, contractile vesicle; *n*, nucleus.

Fig. 10. Minute viviparous forms of *A. villosa*.

Fig. 11. *Amœba princeps* (var. *radiosa*), showing an abortive effort at fission: *c v*, contractile vesicle; *n*, nucleus.

Fig. 12. *Difflugia proteiformis* (var. *septifera*), showing a dithalamous tendency.

Fig. 13. *Difflugia proteiformis* (var. *acuminata*), showing transitionary tendency towards the characters of *Arcella aculeata*; at *c*, the portion of the test around the aperture built up entirely of chitinous pellets. *d*, terminal spine.

Fig. 14. Showing the configuration of the test in *Arcella vulgaris*, consisting of hexagonal depressions, through which the line of fracture generally passes.

Fig. 15. Showing the configuration of the test of *Euglypha* ——? from Stony Stratford; the chitinous pellets taking a perfectly symmetrical form, namely, discoidal masses connected one with the other by regularly disposed bands of the same material. The line of fracture accordingly follows that of the thinnest portion of the test—that is to say, the spaces intervening between the rows of pellets.